

Jay Berzofsky, et al.
Application No. 09/508,552
Page 2

PATENT

Kindly replace the paragraph at page 6, lines 23-28, with the following paragraph:

~~To optimize intrarectal delivery, the immunogenic compositions of the invention also preferably include an absorption-promoting agent, for example a surfactant, mixed micelle, enamine, nitric oxide donor, sodium salicylate, glycerol ester of acetoacetic acid, cyclodextrin or beta-cyclodextrin derivative, or medium-chain fatty acid.~~

Kindly replace the paragraph at page 9, lines 1-8, with the following paragraph:

~~Fig. 11 demonstrates that protection induced by mucosal immunization with HIV-1 peptide vaccine is specific. On day 35, mice were challenged intrarectally with 2.5×10^7 plaque-forming units (pfu) of vaccinia virus expressing gp 160IIIB (vPE16) or with 2.5×10^7 pfu of vaccinia virus expressing β -galactosidase (vSC8). Bars = SEM of five mice per group. The difference is significant at $P < 0.01$ by Student's test.~~

Kindly replace the paragraph at page 11, lines 10-35, with the following paragraph:

~~IR immunization induced long-lasting protective immune responses. For example, antigen-specific CTL were found in both mucosal and systemic sites 6 months after immunization. IR immunization with the antigenic peptide elicited significantly stronger CTL responses than IN immunization with the same peptide. While IR administration with PCLUS3-18IIIB (SEQ ID NO:2) induced a significant response when administered alone, the response was enhanced by the inclusion of CT. The CTL were CD8⁺ T lymphocytes restricted by MHC class I molecules, recognizing MHC class I positive target cells either endogenously expressing HIV-1 gp160 or pulsed with an appropriate gp160 peptide. Induction of both mucosal and systemic CTL response by IR immunization was IL-12-dependent, as shown by inhibition of induction of CTL in mice treated i.p. with anti-IL-12 antibody. Furthermore, inclusion of IL-12 in the composition of antigenic peptide and CT used for IR immunization resulted in enhanced mucosal and systemic CTL responses relative to the responses elicited by antigenic peptide and CT without IL-12. The dependence on IFN γ of mucosal and systemic~~

*Sub
B6*

Jay Berzofsky, et al.
Application No. 09/508,552
Page 3

PATENT

*A4
A concitor*

CTL generation following IR immunization was demonstrated by the absence of such responses in mice which lack the ability to produce functional IFN γ , e.g., as the result of a premature stop-codon in the IFN γ -encoding gene. The stop-codon mutation causes the gene to encode a truncated protein lacking the activity of IFN γ .

Kindly replace the paragraph at page 24, lines 7-21, with the following paragraph:

*Sub
B6
D5*

Accordingly, preferred formulations for administering soluble antigens and CTL-stimulatory cytokines within the methods of the invention are designed to optimize mucosal delivery. These agents may thus include cyclodextrins and beta-cyclodextrin derivatives (e.g., 2-hydroxypropyl-beta-cyclodextrin and heptakis(2,6-di-O-methyl-beta-cyclodextrin). These compounds, preferably conjugated with one or more of the active ingredients and formulated in an oleaginous base, are well documented to enhance bioavailability in intrarectal formulations. Other absorption-enhancing agents adapted for intrarectal delivery include medium-chain fatty acids, including mono- and diglycerides (e.g., sodium caprate--extracts of coconut oil, Capmul), and triglycerides (e.g., amylodextrin, Estaram 299, Miglyol 810).

Kindly replace the paragraph at page 39, lines 21-32, with the following paragraph:

*Sub
B6
JL*

Immunization Mice were immunized with 4 doses of the synthetic HIV peptide vaccine construct PCLUS3-18IIIB (Ahlers et al., *J. Immunol.* 150:5647-5665, (1993)) (50 μ g/mouse for each immunization) on days 0, 7, 14 and 21 in combination with cholera toxin (CT) (10 μ g/mouse) (List Biological Laboratories, Campbell, CA) by intrarectal administration. For subcutaneous immunization, incomplete Freund's adjuvant was used. rm IL-12 (a generous gift of Genetics Institute, Inc., Cambridge, MA) was delivered either intraperitoneally (IP) (1 μ g) or intrarectally (1 μ g) mixed with DOTAP (Boehringer Mannheim), a cationic lipofection agent, along with the peptide vaccine.